Different effects of inhibitors of protein synthesis on the inhibited and augmented LH response of pituitary glands from ovariectomized rats to LH-RH in vitro after pretreatment with oestradiol-17β-benzoate in vivo

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Received 24 June 1982

LH release *Protein synthesis* *Pituitary gland* *Oestradiol-17β-benzoate* *LH-RH* *Ovariectomy*

1. INTRODUCTION

Exposure of pituitary glands to oestrogen results in time-dependent changes of the luteinizing hormone (LH) response to luteinizing hormone releasing hormone (LH-RH): an initial phase of depressed responsiveness (negative phase) during the first 2–3 h is followed by an augmented responsiveness (positive effect) after ~2 h [1] (review [2]).

The effects of oestrogen on their target organs are mediated through RNA and/or protein synthesis dependent steps [3,4]. With regard to both effects of oestrogen on the pituitary LH response to LH-RH, however, there is little conclusive evidence for the involvement of similar mechanisms of action. This is explicable because also LH-RH-induced release of LH from pituitary glands of intact rats is partly dependent upon synthesis of RNA and protein [1,5].

Previous studies demonstrated that LH-RH-induced LH release from pituitary glands in vitro became independent of protein synthesis when the glands had been pre-exposed to either high endogenous or exogenous LH-RH levels [1,6,7]. The use of pituitary glands from ovariectomized (OVX) rats from which LH release normally is independent of protein synthesis, in any case after pretreatment with LH-RH, therefore provides a suitable experimental model to investigate to what extent actions of oestrogens themselves are dependent on protein synthesis. For the experiments OVX rats were injected with oestradiol-17β-benzoate (OB) 2.5 h or 3 days before decapitation and subsequent incubation with LH-RH and inhibitors of protein synthesis.

2. EXPERIMENTAL

Adult female rats from the Wistar-derived colony kept in this laboratory were ovariectomized 14 days prior to decapitation and injected with oestradiol-17β-benzoate (Organon; 7 μg in 0.2 ml arachid oil), or with an equal volume of vehicle only, at 2.5 or 72 h (i.e., 3 daily injections) before decapitation. Then two pituitary halves from different but similarly pretreated animals were placed in flasks each containing 1 ml medium TC 199 (Difco Lab.). The preincubation of 0.5 h in the same media and the successive incubation(s) with fresh media were carried out at 37°C under continuous shaking and gassing with O_2 and CO_2 (95%:5%). LH-RH (Beckman; 1000 ng/ml) was present at a maximally active concentration; cycloheximide (Bochringer; 25 μg/ml) or puromycin (Boehringer; 54 μg/ml) inhibited synthesis of protein for 90–95% within 0.5 h [1]. If cycloheximide or puromycin was to be present during the first incubation period, they were also dissolved in the preincubation medium. Samples of 50 μl medium were withdrawn during and at the end of each incubation period. The LH content of the media and that of the pituitary glands after saline extraction were estimated by radioimmunoassay [1,8] and were expressed as μg LH-RP-1/pituitary gland.

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The reference and the iodination (LH-I-6) preparations were generous gifts from Dr A.F. Parlow and the NIAMDD. Specific rabbit anti-ovine LH was a generous gift from Drs J. Dullaart and J.Th.J. Uilenbroek (Erasmus University, Rotterdam). Statistical comparisons were made by analysis of variance followed by Duncan's multiple comparison test [9].

3. RESULTS

3.1. *Inhibitory effect of OB*

The animals received a single injection of OB or vehicle 2.5 h before decapitation, after which the pituitary glands were incubated for 4 h. Table 1 shows that this pretreatment with OB had no significant effect on basal release of LH. Furthermore, neither cycloheximide nor puromycin affected the spontaneous release of LH significantly. However, this short-term treatment with OB (see fig.1) resulted in a depressed response to LH-RH. Cycloheximide and puromycin failed to affect LH-RH-induced release of LH from oil-treated con-

Table 1

Basal release of LH by pituitary glands of OVX rats [mean LH content of the media, (μg LH-RP-1/pit.) ± SEM ($n = 4$)] after 4 h incubation

Injection	Additions	Time before decapitation	
		2.5 h	3 days
Oil	None	12.5 ± 0.2	10.0 ± 0.4[a]
Oil	Cycloheximide	12.4 ± 0.9	13.4 ± 0.9
Oil	Puromycin	12.7 ± 0.8	11.4 ± 1.4[a]
OB	None	13.4 ± 0.4	16.0 ± 0.5
OB	Cycloheximide	12.5 ± 0.5	17.4 ± 2.1
OB	Puromycin	13.6 ± 0.2	16.8 ± 1.0

[a]Analysis of variance $p < 0.05$ *vs* OB-treated groups

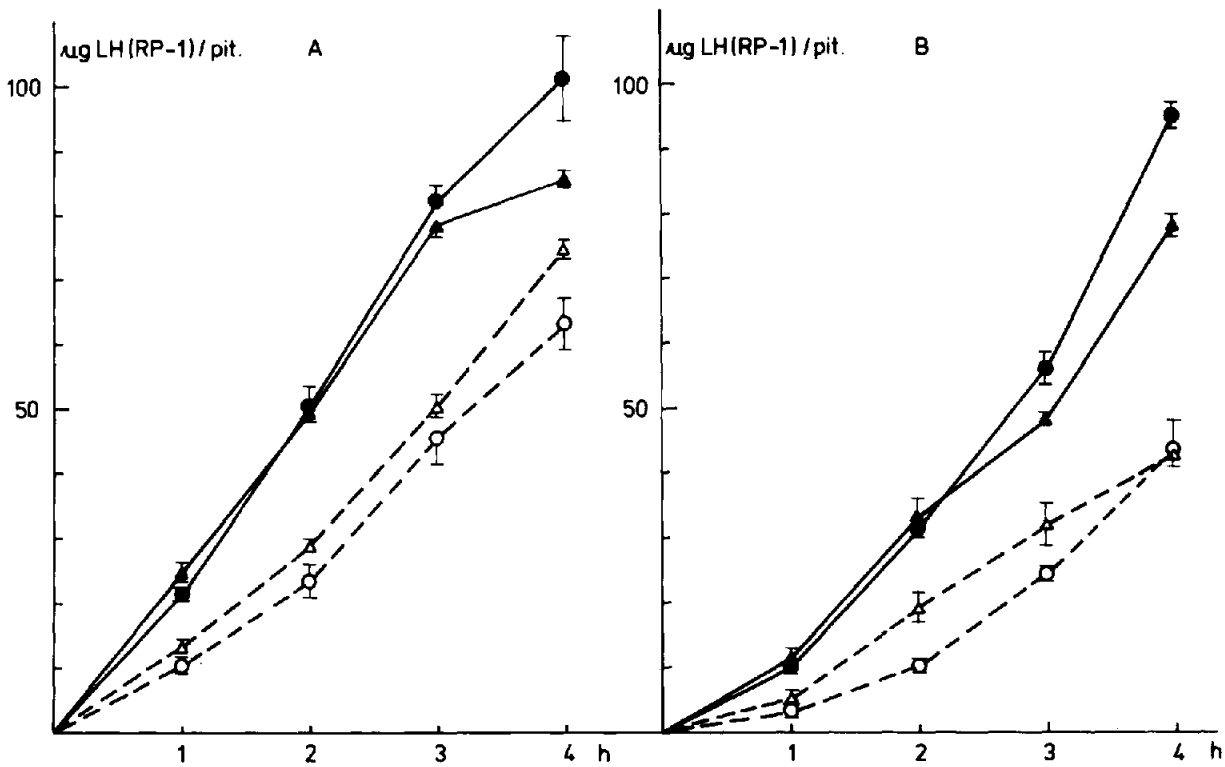

Fig.1. Effect of puromycin (A) or cycloheximide (B) on the LH-RH-stimulated release of LH during incubation of pituitary glands from OVX rats after injection of OB (---) or vehicle (——) 2.5 h prior to decapitation. The results are expressed as means ± SEM, $n = 4$. Additions to the media: LH-RH (●,○) or LH-RH + cycloheximide or puromycin (▲,△).

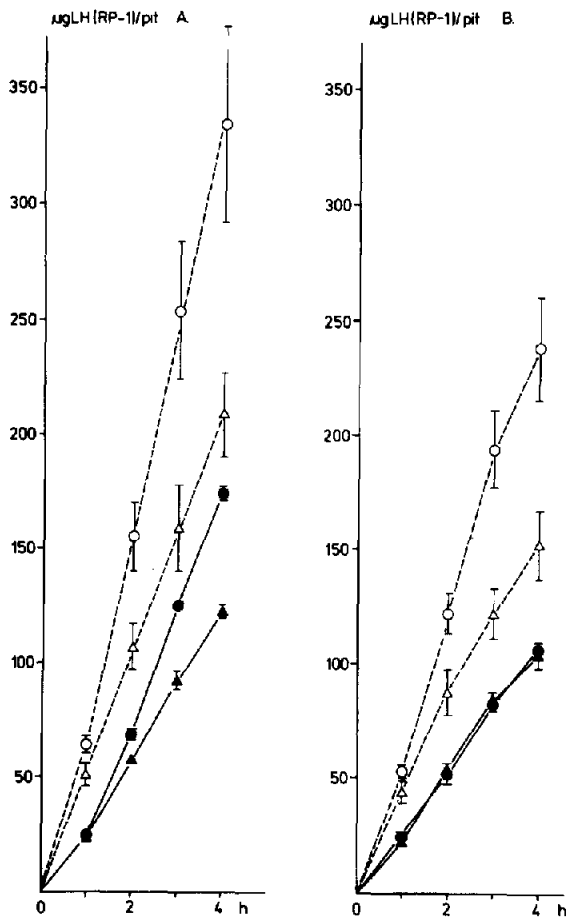

Fig.2. Same legend as for fig.1, except that the rats were pretreated with OB during the 3 days prior to decapitation.

trols, except at the fourth hour of incubation. Moreover, the inhibitory effect of OB on pituitary responsiveness to LH-RH was not, or only slightly, reversed by puromycin or cycloheximide, respectively.

3.2. *Augmentative effect of OB*

The animals received three daily injections of either OB or vehicle prior to decapitation. Contrary to [10] basal release of LH (see table 1) was increased by pretreatment with OB, but neither cycloheximide nor puromycin had any significant effects on this increase. As fig.2 shows, long-term treatment with OB resulted in the well-known enhanced responsiveness of the glands to LH-RH. Both inhibitors of protein synthesis depressed this increased responsiveness. However, whereas cycloheximide had no effect on LH release from pituitary glands of the oil controls, puromycin inhibited this release significantly after 3–4 h incubation.

These inconsistent results hamper in discriminating whether putative protein synthesis dependent actions of LH-RH and/or of OB were inhibited by the antibiotics. Moreover, since it may be assumed that injections of OB in rats also decreased endogenous release of LH-RH (see also [11]), the observed decay in the rate of LH release from glands from OB-pretreated rats might have been caused by a fast depletion of endogenous LH-RH-induced factors related to the synthesis of protein [1,7]. These problems can be overcome by pre-exposure of the pituitary glands to a high concentration of LH-RH only for 2 h, since then subsequently LH-RH-induced release of LH has become independent of further synthesis of protein for at least 4 h of incubation [1,6,7].

Therefore, the experiment depicted in fig. 2 was repeated, but now the incubation was preceded by a 2-h preincubation of the glands with LH-RH only. The results (fig.3) show that now neither cycloheximide nor puromycin had any effect on LH-RH-induced LH release from glands of oil controls, whereas both agents suppressed the augmentative effect of OB on this release. From the course of the curves in fig.2,3 it is also clear that the inhibitory actions of cycloheximide and puromycin took ~ 1 h to develop and became complete after 2 h. Consequently, these results demonstrate

Table 2

LH content of pituitary glands of OVX rats which, 2.5 h or 3 days prior to decapitation, were injected with OB or vehicle

Pretreatment in vivo	Mean LH content [(µg LH-RP-1/pit) ± SEM (n = 4)]
OB, 2,5 h	836 ± 68
Oil, 2.5 h	923 ± 40
OB, 3 days	976 ± 51
Oil, 3 days	879 ± 39

Analysis of variance $p > 0.05$

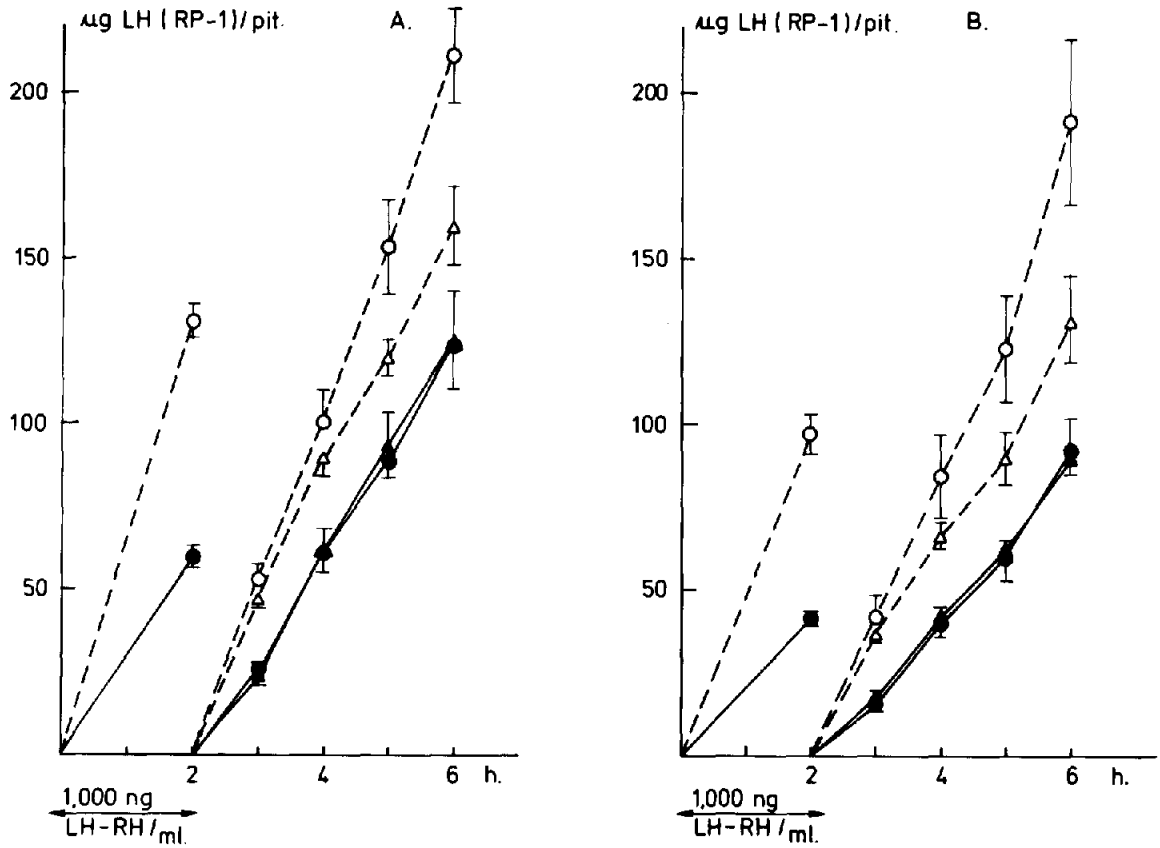

Fig.3. Same legend as for fig.2, except that a 2-h preincubation of the glands with LH-RH (•,○) only was introduced.

that continued, oestrogen-related synthesis of protein is obligatory for prolonged expression of the positive effect.

Finally it was established whether changes of pituitary LH content caused by treating the rats with OB might contribute to the effects observed. Therefore, pituitary LH contents were measured immediately after decapitation. Table 2 shows that pretreatment with OB did not affect pituitary LH content significantly.

4. DISCUSSION

As pointed out in sections 1 and 3, the effects of cycloheximide and puromycin on LH-RH-induced release of LH during incubation must be caused by inhibitory effects on oestrogen-induced synthesis of protein and not on that of LH-RH.

Using a complete in vitro design with pituitary glands from untreated intact or OVX rats we demonstrated that oestradiol-17β added to the incubation medium inhibited the LH release stimulated by LH-RH and, moreover, that the development of this inhibition could be prevented by inhibitors of protein synthesis [12]. The present results show that once the negative effect of OB has developed, inhibition of protein synthesis almost completely fails to reverse the action of oestrogen on LH-RH-induced LH release. Hence, these results implicate that although the induction of the inhibitory action of oestrogen requires synthesis of protein, its maintenance is independent of further synthesis of protein.

In the case of the augmentative effect of oestrogen on the pituitary LH response to LH-RH it was observed that inhibition of RNA [13] or of protein synthesis (fig.2,3) suppressed this effect, in the latter case even within 2 h. Given that at the time of

decapitation the glands had already developed the positive effect, it must be concluded here that continued synthesis of protein is necessary.

The exact mechanism through which oestradiol finally modulates the LH response of the pituitary glands to LH-RH is still unknown. Probably we must exclude changes in LH-RH receptor population to be responsible, since no correlation was observed between LH-RH binding capacity of pituitary glands or receptor affinity and the LH response of the glands to LH-RH 3–24 h after injection of oestradiol [14,15]. Neither the negative nor the positive phase are the result of changes in pituitary LH content [1,16]. Although it has been found that oestrogen pretreatment of pituitary glands may stimulate synthesis of LH [17], our results demonstrate that LH synthesis is not obligatory for the development of the positive phase. However, further maturation of the LH polypeptide chains to the glycoprotein molecule still might play a role [18].

Another intriguing question which remains unsolved is whether both effects of oestrogen on the pituitary LH response are dependent upon each other or whether they may result from separate mechanisms with different time lags. We have already shown that as it did with LH-RH, short-term pretreatment with OB also depressed the response in vitro to elevated K^+ levels. However, after prolonged pretreatment with OB no augmented response to the latter secretagogue developed [10]. Therefore, these and the present results suggest that the negative and positive effects of oestrogen may have been generated, at least partly, through separate pathways, the ultimate responsiveness of the pituitary gland to LH-RH being the result of both mechanisms of action.

ACKNOWLEDGEMENT

This work was partly supported by a grant from the Netherlands Foundation for Medical Research (FUNGO/ZWO).

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